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Implementing the 3Rs in Neuroscience Research: A Reasoned Approach

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The 3Rs—replacement, reduction, and refinement—are aimed at minimizing the welfare costs to animals used in research. Some neuroscientists fear that implementing the 3Rs will prohibit essential studies. Others view them as fundamental ethical principles that improve the quality of research. A regulatory system that integrates science and welfare is most likely to deliver public confidence.

Introduction

Scientists around the world are wondering how changes in regulations governing animal research will influence progress in neuroscience. How, for example, would active promotion of the 3Rs, as required in the new European Directive (European Commission, 2010) and the most recent edition of the Guide for the Care and Use of Laboratory Animals—"the Guide"—(National Research Council, 2011) impact innovative research in neuroscience if applied globally?

The principles of the 3Rs—replacement, reduction, and refinement—were first articulated over 50 years ago by W.M.S. Russell and R.L. Burch (Russell and Burch, 1959). *Replacement* refers to methods that avoid the use of animals either absolutely (e.g., using computer modeling or human volunteers) or relatively (e.g., using invertebrates such as *Drosophila* and nematodes, or cultured cell lines derived from animals). *Reduction* occurs when researchers obtain comparable levels of information from fewer animals, often through improved experimental design and technique or statistical analysis. Where the use of animals is unavoidable, *refinement* refers to improvements to scientific procedures and husbandry that minimize pain or distress and improve welfare.

These principles have long been recognized by Ethical Review Committees and Institutional Animal Care and Use Committees as pivotal to their consideration of research protocols. As stated above, they are highlighted in the Guide as well as the EU Directive, both of which act as key references for animal care and use worldwide. But the manner and extent of implementation of the 3Rs in animal-based research generally, and neuroscience in particular, vary considerably, and not all neuroscience investigators regard the 3Rs as either helpful or binding, much less as appropriate standards to be applied internationally.

Since the ever-increasing globalization of scientific inquiry is leading to both greater collaboration and greater competition among scientists world-wide, a clear, consistent and balanced approach to the use of animals in research is becoming more necessary. Variation in practice in the use of animals makes collaboration, the pooling of data and copublication of results more difficult. And it would surely be unacceptable if cutting ethical corners in the use of animals could give scientists an edge in competition with rival groups. Harmonization is called for, but the debate is whether this requires internationally mandated research standards and policies in relation to principles such as the 3Rs.

Put simply, is there a need for neuroscientists internationally to promote implementation of the 3Rs? And would this limit or enhance the quality of their science?

Differing Views

Some neuroscientists urge caution in considering harmonization of any standards applied to the use of animals in science. They argue that shared principles already exist across the scientific community and that, while there is value in identifying areas of agreement, a divergence of practice within a strong overall ethical framework is desirable. Proper evaluation and reporting on the outcome of such diverse practices could be the optimal route to best practice.

While the goal of the 3Rs serves as a worthy guideline for all neuroscientists, some researchers caution against embedding the 3Rs as a core value, perceiving a risk of raising false and unrealizable expectations or possibly jeopardizing important medical progress.

On the other hand, some neuroscientists take the view that a key consideration for the animals used in their research is to minimize pain and distress and to improve well-being in accordance with their science. The 3Rs are seen by them as a powerful mnemonic for ethically appropriate behavior.

This broad spectrum of views within the neuroscience community reflects the range of interpretations held by the authors of this opinion piece. However, we have become convinced of the value of sharing these views in a transparent manner and, through dialog, moving toward common ground.

A Rational Approach to the 3Rs

It is surely indisputable for any reasonable person that every effort should be made to adopt alternatives to animal experimentation, especially where those alternatives are of proven validity. But it would be wrong to raise expectations that widespread "replacement" of animal models, especially in neuroscience, is feasible in

the foreseeable future. Given our current state of knowledge, it is difficult in the short term to imagine effective research on such topics as the detailed organization of neuronal circuitry, the mechanisms of perception, decision making, learning, memory and attention, the development of the nervous system, the molecular and cellular basis of disease processes, and the repair of damage in the nervous system, without the direct use of animals or preparations derived from animals.

Even the more modest goal of "reduction" in the numbers of animals used in research has to be expressed in a way that does not risk stifling crucial research. For example, the development of ever more sophisticated techniques for modifying genetic function has enabled the creation of much more valuable animal models for the exploration of both normal function (e.g., memory formation) and disease (e.g., neurodegenerative disease). But their very value means these models are likely be used in greater numbers. It is notable that the general increase in numbers of laboratory animals used in the UK over the last 15 years is largely accounted for by increased breeding and use of genetically modified animals—mainly mice and fish. Use of unmodified (wild-type) animals has remained relatively stable in spite of significantly increased public and private investment in biomedical research over this period, which might have been expected to elevate the numbers proportionately.

It is clear that "refinement" deserves much more emphasis. Improvements in husbandry, veterinary care, environmental enrichment, and experimental techniques in neuroscience research have the potential to reduce the ethical cost of research significantly, in terms of suffering, and at the same time improve the quality of the science (e.g., Prescott et al., 2010). There can be little doubt that healthy, contented, unstressed animals make better, more reliable models for scientific research.

Forum on Animal Research

Worldwide changes in the regulatory environment are ongoing and they are certain to have an impact on neuroscience research and related animal care programs. It was in this context that the Institute of Medicine's Forum on Neuroscience and Nervous System Disorders held a workshop at The Royal Society's Chicheley Hall in the UK in July 2011 entitled "International Animal Research Regulations: Impact on Neuroscience Research." Cochaired by Colin Blakemore of the University of Oxford and Arthur Sussman of the MacArthur Foundation, this two-day workshop convened a group of experts and stakeholders—from academia and industry, to law and journalism, from the United States and Europe, to South America and Asia—to exchange ideas and confront the evolving standards and policies head-on and candidly.

The discussants considered not only the various regulatory systems that govern animal care and use, but also the emergence of private party actions to intervene in the enforcement of regulations and the increasing use of freedom of information approaches, such as federal and state "sunshine" legislation in the United States, to seek information about animal care and use (Institute of Medicine, 2012).

Key Messages Emerge

While not representing a total consensus of all the workshop's participants, some important messages emerged during the presentations and subsequent discussions. Key among these was the need for a strong regulatory and institutional compliance framework to ensure that the use of animals in research is ethically secure and legally sound and to provide confidence in public communication about, and defense of, the research. At the same time, delegates were concerned to avoid placing unnecessary constraints on important neuroscience research.

The scientific study of living organisms is critical if we are to understand both life on earth and the diseases and disorders that we cannot yet treat or prevent. Since all living organisms have a common origin and all vertebrates share a large fraction of their genes and a wide range of cellular mechanisms, we have already learned a great deal about the principles of human biology and behavior from animal models and can hope to learn more. Moreover, advances in veterinary care also depend crucially on understanding gained from the study of animals.

A common feature of animal research legislation around the world is that animals may be used for some experimental procedures that would not be acceptable in humans. These include manipulation of the environment, the genetics, or the bodies of the animals.

Nevertheless, it must be appreciated that the use of animals in neuroscience research raises particularly sharp ethical issues. The fact that many harrowing disorders of the nervous system, such as dementias, Parkinson's disease, and motor neuron disorders, are increasing in prevalence and are not adequately treatable heightens the potential benefits of such research. But for the same reasons, neuroscience research often involves the creation of such distressing conditions in animals, or the manipulation of their experience, in ways that highlight the potential ethical costs of animal research.

As neuroscience research moves forward, there is likely to be a continuing reliance on animal models. This likelihood must not be concealed in discussions with politicians, the media, the public, or with groups that oppose animal use. But this should not preclude grasping opportunities to implement the 3Rs: indeed any continuing need to use animals simply raises the moral imperative to optimize welfare and to search for every way to reduce suffering. Indeed, in the next few years mathematical and computational modeling of the function of neurons and neural circuits will become more effective; use of "lower" species such as zebrafish, strengthened by evidence of conservation of genetic mechanisms, is likely to become even more powerful; and advanced imaging techniques will help to reduce numbers of animals used and to refine the nature of studies.

Core Principles, Not Uniform Rules

Many researchers (and ethicists) consider that the application of core guiding principles for animal care and use is preferable to the application of slavish general rules. Such principles include the following:

(1) defining the needs and promises of neuroscience research—asking critically whether animals are the optimal and justifiable model and what discoveries are likely to result from their use in the laboratory;
(2) making the care and welfare of all research animals a high priority—scientists must be certain that the animals they use are treated

humanely, above all for ethical reasons, but also to optimize the health and behavioral status of the animals and hence the validity and consistency of research results;

(3) striving to "replace, reduce, and refine" animal use wherever possible; and

(4) securing public confidence that oversight of research is sufficiently rigorous but mindful of, and informed about, the linkage between use of animal models and medical progress.

Through rigorously applying these core principles, scientists, regulators, and other stakeholders can best collaborate to develop transparent and workable criteria that reflect the interests of the public and patients in both animal welfare and scientific progress. Many advocate an approach that takes into consideration both the welfare of the animals and the quality and potential benefits of the research in a "cost-benefit analysis" (Animal Procedures Committee, 2003). At the same, time they urge that while the regulatory framework should ensure compliance by investigators and institutions, it should also avoid imposing undue bureaucratic burdens.

Society Seeks a Balanced Approach

The problem of improving our understanding of living systems and their disorders remains, and the ethical care and use of research animals are critical to that understanding. We must consider our commitment to animal welfare in the context of important scientific goals together with both the needs and concerns of society (Figure 1). The magnitude of the challenges of neuroscience research, and especially the growing and costly toll of diseases of the nervous system around the world, must be prominent in the minds of all who have an interest in the conduct of medical research.

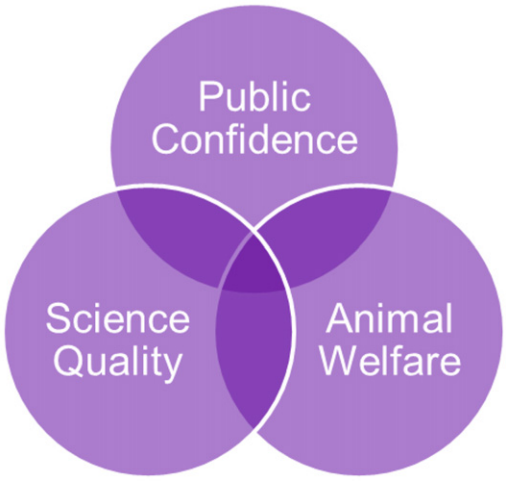

Figure 1. A Model of Regulatory Balance in Animal Research

In any regulatory system, it is vital to ensure that bureaucracy does not become so burdensome as to inhibit scientists from developing important research proposals. However, it is equally important to ensure that animals suffer no more than necessary. A balance is demanded between the needs of the science and the needs of the animals, and it is this balance which commands public confidence in the regulatory system. The public wants to benefit from scientific advances but also to be reassured that animals are not suffering unnecessarily (reprinted with permission from MacArthur Clark, 2012).

Given the complexity of some of these arguments and the apparently seductive appeal of efforts to curtail the use of animals in science, it becomes both a necessity and a duty for neuroscientists to listen to public concerns and to reach out to inform and engage the public, including those with a professed concern for animal welfare, about why this research is important. Neuroscientists need to become skilled at explaining, in lay terms, how the animal models that they select are the least distressing and the most likely to promote scientific advances that will benefit all living beings. The objective should be to achieve maximum benefit from the minimum number of animals while causing the least pain or distress.

Consideration and implementation of the 3Rs must therefore be thoroughly integrated into the procedures for the approval of all animal research protocols. Importantly, Russell and Burch viewed the implementation of the 3Rs as a means of improving the quality of science, not merely as a measure toward improving welfare. The 3Rs should be used to effectively reduce the potential harms of proposed research so that science with potentially significant benefits can be properly justified in the public eye.

It is clear that neuroscientists must recognize the importance, both symbolic and real, of "replacement, reduction, and refinement" whenever animals are used. However, they may be most persuaded of this through realizing that rational implementation of the 3Rs will improve their science and help enable them to strive for "relevance, robustness, and reliability" in their investigations. The IOM Forum was a useful step in the honest and nuanced dialog that must continue as scientists, lawmakers, regulators, welfare organizations, and the public define the path forward for realizing the huge potential of neuroscience while supporting the proliferation of sensible, ethical, and balanced legal and regulatory systems.

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